wanted to further investigate this relationship in situ on articular cartilage from patients with knee OA by measuring the telomere length in chondrocytes from defined areas of the tibia plateau. Other groups before us have measured the mean telomere length in OA cartilage but a large body of evidence now suggests that it is the shortest telomeres in the cell that – when they becomes critically short – activates the cellular surveillance systems and send the cell into senescence. Therefore it seems evident that the mean telomere length is a less reliable measure when telomere-associated senescence is imminent. Therefore we instead measured the shortest telomeres in the cartilage and compared the degree of shortness to the severity of OA. For that purpose we used a modified version of the STELA method, which measures the 15−30 shortest telomeres present in a DNA sample. By correlating the results with the degree of cartilage degradation established through histopathological scoring of parallel sections, we have obtained paired data that provides us with a tool to compare the chondrocyte telomere length with the severity of OA in different regions of the cartilage.
Methods: Human articular cartilage was obtained from OA patients undergoing total knee replacement surgery. Cartilage samples from defined areas of the tibia plateau were collected and tissue sections for histopathological grading were prepared. STELA – a PCR based method for measuring telomere length – was performed on DNA isolated from corresponding areas of the cartilage. The telomeres were detected with southern blotting and the fraction of shortest telomeres was calculated. Grading of parallel sections for severity of OA was done with Mankin Histologic/Histochemical Grading System.
Results: The results show a significant correlation between OA severity and the fraction of short chondrocyte telomere lengths. The fraction of short telomeres increases as the level of cartilage degeneration increases closer to OA lesion sites.
Conclusions: We have confirmed a relationship between short telomeres and severity of OA by finding a significant correlation between the fraction of short telomeres and the histopathological score for OA. This study thus lays the ground for further work aiming at investigating the role of telomere shortening in the development of OA.

207 THE TIDEMARK OF CARTILAGE: A DEPOT FOR APOPTOTIC DEBRIS? A TARGET FOR AUTOIMMUNE DISEASE?

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Purpose: The tidemark of adult articular cartilage lies at the interface between its deep, calcified and uncalcified zones. Previous authors have found that this narrow trilaminar band is recognized by a diverse array of staining techniques, but none have offered a plausible mechanism for its histologic promiscuity. We hypothesize that it reflects a corresponding molecular diversity as an extracellular deposit for the debris from apoptotic chondrocytes. If so, articular cartilage is unique among human tissues for its extracellular display of otherwise privileged intracellular antigens and this property may explain the articular targeting of systemic autoimmune diseases.
This work aims ultimately to characterize the constituents of the tidemark and to determine whether one or more of them may serve as targets for autoimmune, inflammatory disease.
Methods: Normal, metacarpophalangeal joints from cadavers donated for anatomic study were examined as unfixed frozen, methacrylate embedded, and acid-decalcified/paraffin embedded tissue. Sections (4−8 micron) were evaluated with standard histologic stains and with indirect immunofluorescence.
Results: In this preliminary report, we add DAPI (considered specific for DNA) and Masson Trichrome (often used for actinomyosin) to the 20 stains previously reported to recognize this distinctive band. Their specific targets include fat, enzymes, unidentified cytoplasmic proteins, and lectins. Many of these diverse solutes should be charge-excluded from the highly anionic matrix of normal cartilage, but each of them may be a chondrocytic constituent that is released upon apoptotic death. None of the positive stains found a concurrent deposit at the nearby interface between calcified cartilage and bone, thus their presence in the tidemark reflects recognition of molecular deposits rather than a nonspecific surface effect.
Conclusions: These findings are consistent with the hypothesis that the chondrocytes of cartilage are replaced regularly (albeit slowly) from resident stem cells, and that they move through the matrix to an ultimate apoptotic death. Loading forces then drive their debris downward until it is arrested by a semipermeable barrier of calcified cartilage. This deposit, the tidemark of normal cartilage, would then be an extracellular deposit of citrullinated, intracellular antigens that could fuel the fires of those systemic, autoimmune diseases that attack synovial joints.

208 ACUTE CHONDROCYTE DAMAGE IN HUMAN ANKLE INTRAARTICULAR FRACTURE

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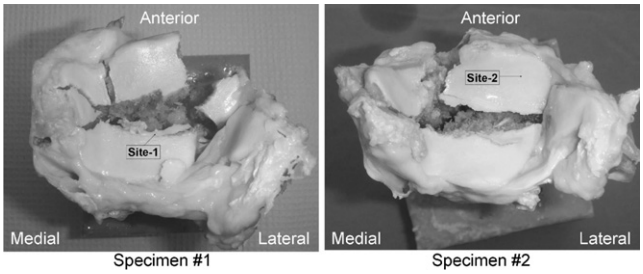
Purpose: It has been recognized that intraarticular fractures (IAFs) typically involve acute cartilage damage, such as death/dysfunction of chondrocytes and disruption of interstitial matrix. This acute cartilage damage presumably initiates a pathologic cascade leading to joint degeneration. However, information about fracture-associated chondrocyte damage in acute human injuries is very limited. Various rates of chondrocyte death in fracture fragments (0 to 50%) have been reported in the literature. Unfortunately, those data were from small fracture fragments retrieved at open reduction surgeries, and their cartilage pathology would likely have been modified during the duration from injury to surgery, by natural biological responses such as by inflammatory cytokines. The aim of the present study was to document cell-level cartilage pathology directly associated with acute mechanical damage by a fracture event itself.
Methods: Two human ankles immediately following surgical amputation, from femoral malignant tumor patients (in their 40s and 50s), were subjected to a quasi in vivo fracture impact experiment. The specimens were dissected at the middle of tibial shaft and the subtalar joint, and both ends were potted into PMMA blocks. Each ankle was mounted on a drop-tower, up-side down. The tibial shaft was secured perpendicularly to the base plate, while the talar block was held so that ankle position could be kept neutral. A mass with 60 joules of potential energy was then dropped onto the flat plantar surface of the talar block. Both tibial and talar articular surfaces were sampled immediately, and stored in culture medium for 8 hours (to permit chondrocyte death resulting from mechanical damage.) These samples were subjected to viability analysis using a confocal microscope. Staining with Calcein-AM/Ethidium Homodimer, the superficial surfaces and cross sections at several sampling points for each ankle were scanned, and live/dead cells were counted. The tibial sampling points were near the fracture edges and at the middle of non-fracture area of the anterior and posterior fragments, while the talar sampling points were at the middle of the superior talar dome surface.



Results: In both ankles, experimental IAFs occurred on the distal tibial surface (Figure). In the tibial superficial scans, appreciable chondrocyte death (cell death rate higher than 10%) was identified at only two sites: near a relatively severely damaged fracture edge of the posterior fragment in Specimen #1 (Site-1, 37.1%), and at the middle of the anterior fragment in Specimen #2 (Site-2, 15.4%). Superficial cell death rates at the other 7 sites on the tibial surface were 3.0±3.6% (mean±SD), and those measured on the talar surface were 1.1±0.2%. Cell death rates in the cross sectional scans (including both tibial and talar data) were 4.6±4.2%.
Conclusions: Morphologically, the fractures created were very similar to clinical pilon fractures, suggesting that the natural injury mechanism of human ankle IAFs was reasonably replicated in this experimental model. Inconsistency in cell death rates across surface suggests potential site-specificity in fracture-associated cartilage damage. The overall cell death rates in this study were much lower than those in metal-platen blunt impact experiments ex vivo (typically >40%), implying striking difference in the mechanisms of cartilage damage between IAFs and experimental blunt impact injuries. Discrepancy of cell death rates, as compared with the clinical fracture fragment data, may be associated with delayed apoptosis and/or subsequent damage due to biological responses in clinical IAFs.